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CONFIRMATION

European Patent Office
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Germany

To telefax No. 0049 89 2399-4465 - Confirmation by mail

11 February, 2005

PCT Application No. PCT/DK2004/000259
Kræftens Bekæmpelse
Agents ref.: P734PC00 – LAD

Dear Sirs

Enclosed please find a demand for preliminary examination in respect of the above PCT application. The handling fee and examination fee as specified in the fee calculation sheet will be debited from our account No. 28030052.

Response to written opinion of 21 October 2004

Below please find a response to the written opinion of 21 October 2004. With the enclosed amendments it is anticipated that examiner can acknowledge novelty and inventive step of all claims. In the event that Examiner intends to issue a negative preliminary examination report, the applicant requests

- either a telephone interview pursuant to Rule 66.6 PCT
- or a second written opinion.

Claims

Please find enclosed an amended set of claims 1 to 53, replacing originally filed claims 1 to 54

New claim 1 relates to polypeptides comprising one of four specific peptides identified in previously filed claim 1 as well as functional equivalents thereof. Basis for this amendment can be found in previously filed claim 1 as well as in the description on page 56, l.2-17.

Basis for new claim 2 can be found in the description p. 54, l. 7 to p. 56, l. 1.

Basis for amended claims 27 and 39 can be found in previously filed claim 27 and 39, respectively.

Basis for new claim 48 can be found in previously filed claims 48 and 49.

Novelty

Amended claim 27 relates to a vaccine composition comprising isolated polypeptides comprising specific ML-IAP peptides. D1 describes a vaccine composition comprising tumour cells transduced with pMFG-GM-CSF. This vaccine composition does not comprise isolated ML-IAP peptides. On p. 3400, left col., 2nd paragraph D1 discloses that serum from a patient previously vaccinated with intact tumour cells transduced with pMFG-GM-CSF comprises antibodies recognising ML-IAP. Such a disclosure does not amount to a disclosure of a vaccine composition comprising isolated polypeptides comprising specific ML-IAP peptides.

Accordingly, claim 27 is novel over the disclosure of D1.

Claims 51 and 52 (corresponding to previous claims 52 and 53) are directed to T-cells obtained by co-cultivating T-cell with a polypeptide according to claim 1. The polypeptides of claim 1 are not disclosed by D1 and accordingly D1 also does not disclose T-cells obtained by co-cultivation with said polypeptides.

Accordingly claims 51 and 52 are novel over the disclosure of D1.

Inventive step

D1 describes vaccination of a melanoma patient with tumour cells derived from said patient transduced with pMFGH-GM-CSF. The document furthermore describes the identification of antigens recognised by antibodies from the serum of said patient. Furthermore, D1 discloses binding of two ML-IAP peptides to T-cells and induction of IFN-γ production by said peptides.

Specific T-cell responses to the peptides described in D1 were only detectable in a patient that had been subjected to numerous immunisations with tumour cells expressing GM-CSF. Such an extensive treatment may induce responses to peptides, which are not superior T-cell epitopes. In contrast, superior T-cell epitopes, are epitopes against which a spontaneous T-cell response is raised without prior immune therapy (see the present application p. 30, l. 10-17). Thus, a technical problem solved by the present invention is the identification of improved peptides derived from ML-IAP which are capable of eliciting a specific T-cell response.

It is well known to the skilled person that the identification of peptides useful for inducing specific T-cell responses is not a straight-forward task (see for example application p. 7, l. 5-27). Even though peptides capable of inducing a specific T-cell response derived from a particular protein are known, the skilled person cannot based on this information readily identify further useful peptides. Thus, regardless that D1 describes ML-IAP peptides capable of inducing IFN-γ production, the skilled person seeking to identify additional ML-IAP peptides would not have had any reasonable expectation of success. In particular, when seeking to identify improved ML-IAP peptides the skilled person would have had absolutely no reasonable expectation of success. Accordingly, the polypeptides claimed in claim 1 of the present application were not obvious and it required an inventive step to identify the polypeptides. In T149/93 the Technical Board of Appeal of the European Patent Office states that if the skilled person when embarking on a project realises that its successful conclusion depends not only on technical skill but also on the ability to take the right decisions along the way inventive step should be acknowledged. In this context it should be noted that the art of identifying suitable peptides for inducing specific T-cell responses is unpredictable.

The polypeptides claimed in claim 1 are indeed superior to the ML-IAP peptides described in D1. This can be deduced from figure 1 to 3 of the present application. As is evident from figures 1 and 2, the peptides identified by SEQ ID 298 (ML-IAP 280)(see fig. 1), SEQ ID 297 (ML-IAP 245)(see fig. 2, patient FM96) and SEQ ID 301 (ML-IAP 230, see fig. 2, patient FM101) give rise to the strongest spontaneous T-cell responses detected in Tumour infiltrating lymphocytes. Furthermore, at least in one patient each of these peptides give the strongest spontaneous response detected. The peptide identified by SEQ ID 245 gives rise to a very strong spontaneous T-cell response in HLA-A3 antigen positive patients (see fig. 3) and thus provides the first HLA-A3 restricted peptide derived from ML-IAP capable of raising a specific T-cell response.

D2 describes peptides derived from survivin. Similar to ML-IAP, survivin is an inhibitor of apoptosis. However, peptides derived from one inhibitor of apoptosis are of no aid in the identification of useful peptides from a completely different inhibitor of apoptosis. Thus, based on the disclosure of D1 in combination with the disclosure of D2, the skilled person would also not have had any reasonable expectation of success in identifying suitable ML-IAP peptides.

Accordingly, claim 1 indeed is inventive over D1 taken alone or in combination with D2. In addition claims 2 to 13 dependent on claim 1 are inventive as well. Similarly, independent claims 14, 19, 20, 26, 45, 48, 51, 53 and claims dependent thereon are inventive for the same reasons. Dependent claim 28 and claims dependent thereon are also inventive for the same reasons.

Claim 27 is directed to vaccine compositions comprising isolated polypeptides comprising specific ML-IAP peptides. D1 does not describe such vaccines (see herein above). D1 merely suggest that vaccines can incorporate ML-IAP and additional antiapoptotic proteins and other gene products crucial for cancer maintenance. The only example of such vaccines given in D1 is vaccines comprising intact tumour cells.

Based on the disclosure by D1, the skilled person would have believed that vaccines should not comprise isolated ML-IAP peptides, but rather a number of different antigens, such as provided by intact tumour cells.

The skilled person would thus not have been able to prepare a vaccine as claimed in claim 27 based on the disclosure by D1 without the application of inventive skills. In particular the skilled person would not have arrived at vaccines comprising polypeptides comprising the specific ML-IAP peptides claimed in claim 27, because nothing in the disclosure of D1 even hints at vaccines comprising these peptides. These specific ML-IAP peptides are inventive for the reasons outlined herein above.

Examiner is therefore respectfully requested to acknowledge inventive step of claim 27 and claims dependent thereon. Similar arguments apply to claim 39 and claims dependent thereon. In addition, independent claims 37, 41, 44 and claims dependent thereon are inventive for the same reasons.

Clarity

Claims 27, 39 and 48 have been amended to refer to specific fragments defined by their SEQ ID numbers. The claims should thereby be rendered clear.

Yours sincerely
HØIBERG A/S

Louise Aagaard

Demand under article 31 PCT
Fee calculation sheet
Form 1037

Claims

1. A polypeptide fragment capable of raising a specific T-cell response, said fragment comprising a peptide selected from the group consisting rlqeertck (SEQ ID NO:245), rlqeertckv (SEQ ID NO:297), qlcpicrapv (SEQ ID NO:298), vleppgardv (SEQ ID NO:301) and functional equivalents having at least 75% sequence identity thereto; wherein said polypeptide fragment comprises at the most 15 amino acids.

2. The polypeptide fragment according to claim 1, wherein said functional equivalent comprises substitutions only in the preferred positions and only to preferred amino acid residues for a given HLA allele as identified in table 2.

3. The polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises at the most 10 amino acids.

4. The polypeptide fragment according to any of claims 1 to 3, wherein the specific T-cell response is measured as more than 50 peptide specific spots per 10^6 cells in an ELISPOT assay performed without pre-stimulation in vitro.

5. The polypeptide fragment according to any of claims 1 to 3, wherein the specific T-cell response is measured as more than 50 peptide specific spots per 10^5 cells in an ELISPOT assay performed after stimulation in vitro.

6. The polypeptide fragment according to any of claims 1 to 3, wherein the specific T-cell response is measured as more than 50 peptide specific spots per 10^6 cells in an ELISPOT assay performed using PBL from an individual that has not been subjected to immune therapy against a neoplastic disease.

7. The polypeptide fragment according to any of claims 1 to 3, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to a MHC (Major Histocompatibility Complex) class I molecule.

8. The polypeptide fragment according to claim 7, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 1000.

9. The polypeptide fragment according to any of claims 7 and 8, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 100.

10. A polypeptide fragment according to any of claims 7 to 9, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 31.

11. A polypeptide fragment according to any of claims 7 to 10, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 5.

12. A polypeptide fragment according to any of claims 1 to 11, wherein the fragment is capable of activating T-cell growth in vitro.

13. A polypeptide fragment according to claim 12, wherein the fragment is capable of activating T-cell growth in vitro so that more than 10^5 antigen specific CTLs may be harvested after 4 stimulation cycles starting with 10^4 PBMC

14. A method of selecting a peptide comprising a fragment of ML-IAP for use in a vaccine composition comprising the steps of

i) Providing an individual who has not been subjected to immune therapy

ii) Providing a polypeptide fragment comprising a peptide consisting of at least 9 consecutive amino acid residues of ML-IAP (SEQ ID NO:1),

iii) Testing specific T-cell responses against fragments of ML-IAP in said individual

iv) Selecting fragments of ML-IAP wherein said T-cell response corresponds to or is better than a predetermined selection criterium.

15. The method according to claim 14, wherein said peptide is selected from the group consisting of rlqeertck (SEQ ID NO:245), qilgqlrpl (SEQ ID NO:55), ltaevppel (SEQ ID NO:100), gmgseelrl (SEQ ID NO:84), elptprrev (SEQ ID NO:200), rlqeertckv (SEQ ID NO:297), qlcpicrapv (SEQ ID NO:298), llrskgrdfv (SEQ ID NO:300), vleppgardv (SEQ ID NO:301) and pltaevppel (SEQ ID NO:302).

16. The method according to claim 15, wherein said polypeptide fragment comprises at the most 15 amino acids.

17. The method according to claim 14, wherein testing said T-cell response comprises an ELISPOT assay.

18. The method according claim 17, wherein said predetermined selection criterium is more than 50 peptide specific spots per 10^6 cells in said ELISPOT assay.

19. A polypeptide fragment according to any of claims 1 to 13 for use as a medicament.

20. Use of one or more polypeptide fragments according to any of claims 1 to 13 in the manufacture of a medicament for treatment of a clinical condition in an individual in need thereof.

21. Use according to claim 20, wherein said clinical condition is cancer.

22. Use according to claim 21, wherein the cancer is malignant melanoma.

23. Use according to claim 20, wherein said clinical condition is an auto-immune disease.

24. Use according to any of claims 20 to 23, wherein at least one of said polypeptide fragments is restricted to an HLA molecule present in said individual.

25. Use according to any of claims 20 to 24, wherein said individual has not previously been subjected to immune therapy against a neoplastic disease.

26. A medicament for treating a clinical condition comprising a polypeptide according to any of claims 1 to 13 as an active ingredient.

27. A vaccine composition comprising an isolated polypeptide comprising a peptide selected from the group consisting rlqeertck (SEQ ID NO:245), rlqeertckv (SEQ ID NO:297), qlcpicrapv (SEQ ID NO:298), vleppgardv (SEQ ID NO:301) and functional equivalents having at least 75% sequence identity thereto; and a pharmaceutically acceptable carrier and/or adjuvant.

28. The vaccine composition according to claim 27, wherein said composition comprises at least one polypeptide fragment according to any of claims 1 to 13.

29. The vaccine composition according to any of claims 27 to 28 further comprising an adjuvant.

30. The vaccine composition according to claim 29, wherein the adjuvant is selected from the group consisting of Montanide ISA-51 and QS-21

31. The vaccine composition according to any of claims 27 and 28, wherein the composition further comprises a carrier.

32. The vaccine composition according to claim 31, wherein the carrier is a dendritic cell.

33. The vaccine compositions according to claim 27 to 28, wherein the composition comprises more than one different ML-IAP fragment according to any of claims 1 to 13.

34. The vaccine composition according to claim 33, wherein the composition comprises different ML-IAP fragments, wherein said fragments are capable of associating with the most frequently occurring MHC class I molecules.

35. The vaccine composition according to claim 33, wherein the composition comprises at least 2 different ML-IAP fragments each capable of associating with a different HLA molecule selected from the group consisting of HLA-A2, HLA-A1, HLA-A3, HLA-A24, HLA-B7, HLA-B27 and HLA-B44.

36. The vaccine composition according to claim 35, wherein the composition comprises at least one class I-restricted ML-IAP peptide and at least one class II-restricted ML-IAP peptide.

37. A pharmaceutical composition comprising the vaccine composition according to any of claims 27 to 36 and an anti-cancer medicament.

38. The pharmaceutical composition according to claim 37, wherein the anti-cancer medicament is selected from the group consisting of chemotherapeutic agents and immunotherapeutic agents.

39. A kit-of parts comprising comprising a polypeptide comprising a peptide selected from the group consisting rlqeertck (SEQ ID NO:245), rlqeertckv (SEQ ID NO:297), qlcpicrapv (SEQ ID NO:298), vleppgardv (SEQ ID NO:301) and functional equivalents having at least 75% sequence identity thereto; and a bioactive compound selected from the group consisting of a chemotherapeutic agent, an immunotherapeutic agent, and a second cancer vaccine composition.

40. The kit-of-parts according to claim 39 comprising one or more polypeptide fragments according to any of claims 1 to 13.

41. A method for treatment of an individual diagnosed with cancer, said method comprising the step of administering to the individual the polypeptide fragment according to any of claims 1 to 13, or the vaccine composition acording to any of

claims 27 to 36, or the pharmaceutical composition according to any of claims 37 and 38 or the kit of parts according to any of claims 39 and 40.

42. The method according to claim 41, wherein said individual has not previously been subjected to immune therapy against a neoplastic disease.

43. The method according to claim 41, wherein said cancer is malignant melanoma.

44. A method for prophylactic treatment of an individual at risk of developing a cancer, said method comprising the step of administering to the individual the polypeptide fragment according to any of claims 1 to 13, or the vaccine composition acording to any of claims 27 to 36, or the pharmaceutical composition according to any of claims 37 and 38 or the kit of parts according to any of claims 39 and 40.

45. A method for raising a specific T-cell response against an epitope of ML-IAP (SEQ ID NO:1) in an individual, said method comprising the steps of administering to the individual a polypeptide fragment according to any of claims 1 to 13, and raising a specific T-cell response against an epitope of ML-IAP in the individual.

46. The method according to claim 45, wherein the methods comprises administering one or more polypeptide fragments according to any of claims 1 to 13, and wherein at least one fragment is restricted to an HLA molecule present in said individual.

47. An antibody capable of specific recognition of a polypeptide fragment according to any of claims 1 to 13.

48. A method for activating and expanding T-cells specific for ML-IAP or fragments thereof comprising the steps of co-cultivating T-cells and one or more polypeptide fragments according to any of claims 1 to 13.

49. The method according to claims 48, wherein the method comprises generating and loading monocyte-derived dendritic cells (DC) with said polypeptide fragment(s) and co-cultivating said DC and perifiral blood monocytes (PBMC) comprising T-cells.

50. The method according to claim 48, wherein the method comprises generating *Drosphila melanogaster* cells expressing one or more different HLA molecules, loading said *Drosophila melanogaster* cells with said polypeptide fragment(s) and co-cultivating said *Drosphila* cells with perifiral blood monocytes (PBMC) comprising T-cells or T-cells purified from PBMC.

51. ML-IAP specific T-cells obtained by the method according to any of claims 48 to 50.

52. T-cells according to claim 51, wherein said ML-IAP specific T-cells are cytotoxic T-cells.

53. Use of ML-IAP specific T-cells according to any of claims 52 and 53 for the preparation of a medicament for treatment of a clinical condition in an individual in need thereof.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: P734PC00

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/DK2004/000259	7 April 2004 (07.04.04)	11 April 2003 (11.04.03)

Title of invention: Therapeutic cancer vaccine

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/DK2004/000259

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

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Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[X] the international application as originally filed

the description
[ ] as originally filed
[ ] as amended under Article 34

the claims
[ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings
[ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of the applicable time limit under Rule 69.1(d).

4. [ ] The applicant expressly wishes the international preliminary examination to start earlier than at the expiration of the applicable time limit under Rule 54*bis*.1(a).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.

[ ] which is the language of a translation furnished for the purposes of international search.

[ ] which is the language of publication of the international application.

[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

Sheet No. 3

International application No.
PCT/DK2004/000259

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate power of attorney
3. ☐ original general power of attorney
4. ☐ copy of general power of attorney; reference number, if any:
5. ☐ statement explaining lack of signature
6. ☐ sequence listing in computer readable form
7. ☐ tables in computer readable form related to a sequence listing
8. ☒ other *(specify)*: response to the written opinion

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Copenhagen, 11 February 2005

Høiberg A/S
Louise Aagaard; Representative of applicant

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.
   ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.
6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54*bis*.1(a) and item 7 or 8, below, does not apply.
7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54*bis*.1(a) as extended by virtue of Rule 80.5.
8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54*bis*.1(a), the delay in arrival is EXCUSED pursuant to Rule 82.

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Demand received from IPEA on: